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Synthesis of allylic trifluoromethyl ketones and their activity as inhibitors of the sex pheromone of the leopard moth, *Zeuzera pyrina* L. (Lepidoptera: Cossidae)

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Abstract

BACKGROUND: Trifluoromethyl ketones (TFMKs), structurally related to the pheromones, are good inhibitors of pheromone communication in insects. To determine their activity on *Zeuzera pyrina* L. (Lepidoptera: Cossidae), a polyphagous pest, the authors have prepared two diunsaturated TFMK analogues of the major (3) and the minor (4) pheromone components, and two monounsaturated ones (5, 6). Their biological activity in electroantennogram (EAG), wind tunnel and field tests is presented.

RESULTS: The synthetic strategy to obtain the allylic TFMKs 3 and 5 is based on the reactions of diene 10 and 1-octadecene with trifluoroacetaldehyde ethyl hemiacetal, followed by Dess–Martin oxidation of the resulting homoallylic trifluoromethyl alcohols. In EAG, topical application of analogues 3 and 4 on male antennae significantly reduced the pheromone response. In the wind tunnel, compound 4 reduced the number of contacts with the pheromone source. In the field, traps baited with mixtures of pheromone and inhibitors captured significantly fewer males than the pheromone alone.

CONCLUSION: An efficient synthesis of allylic TFMKs is reported, with good overall yield, regiospecificity and diastereoselectivity. These compounds are good inhibitors of the pheromone in electrophysiology, wind tunnel and field tests. The results show the importance of two unsaturations at positions 2 and 13 of the trifluoroacyl group in the structure of the analogues, the latter being critical for inhibitory activity.

Keywords: *Zeuzera pyrina*; leopard moth; pheromone inhibition; allylic trifluoromethyl ketones

1 INTRODUCTION

The leopard moth *Zeuzera pyrina* L. (Lepidoptera: Cossidae) is a worldwide distributed wood borer, mainly present in the southern regions of Europe, Asia and North Africa. The larvae seriously damage pear, apple, hazel, cherry and olive trees by excavating galleries in shoots, branches and the main trunk that can eventually cause the death of the tree. Its sex pheromone, emitted by adult females, is a mixture of (*E*,*Z*)-2,13-octadecadienyl acetate (**1**), (*E*,*Z*)-3,13-octadecadienyl acetate (**2**) and (*Z*)-13-octadecenyl acetate in a 78–86 : 4:18–10 ratio (Fig. 1).[1–3] A 96 : 4 mixture of compounds **1** and **2** is used for male flight monitoring, and for population control by mass trapping and mating disruption. Both techniques have been applied successfully in several countries, but the best results have been achieved by mating disruption.[4–7] Trifluoromethyl ketones (TFMKs) are known to inhibit a number of esterases and proteases, such as acetylcholinesterase, chymotrypsin, human liver carboxylesterases[8,9] and, particularly, the antennal esterases present in insect olfactory tissues.[8,10–12] TFMKs act through the formation of a stable hemiacetal of tetrahedral geometry with a serine residue of the enzyme.[13,14]

Following work aimed at developing pheromone perception inhibitors of insect pests,[15–17] the authors report in the present paper on the chemical synthesis and the inhibitory activity of TFMKs **3** to **6** (Fig. 1), analogues of the pheromone components of the leopard moth *Z. pyrina*, in electrophysiology, wind tunnel and field tests. TFMKs **3** and **4** are analogues of the major (**1**) and of the minor (**2**) components of the pheromone respectively. TFMKs **5** and **6** are analogues of both components, with only one double bond; they were synthesised and tested in order to ascertain the importance of the double unsaturation. Particularly interesting are the allylic TFMKs **3** and **5**, for which some of the

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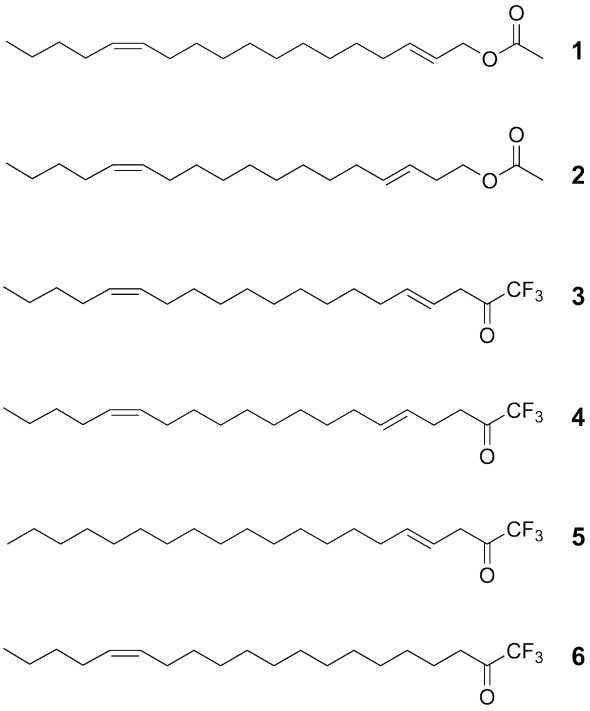

Figure 1. List of chemical structures.

known synthetic methods for TFMKs have proved unsuitable.[18–20] An efficient methodology is presented for obtaining allylic TFMKs, based on the addition of terminal olefins to trifluoroacetaldehyde ethyl hemiacetal in the presence of a Lewis acid as catalyst.

2 EXPERIMENTAL

2.1 General

The pheromone blend (pheromone, hereinafter) containing a mixture of (*E*,*Z*)-2,13-octadecadienyl acetate (**1**) and (*E*,*Z*)-3,13-octadecadienyl acetate (**2**) in a 96 : 4 ratio, (*Z*)-13-hexadecenal, (*Z*,*E*)-3,13-octadecadienol and (*Z*)-11-hexadecen-1-ol were kindly provided by SEDQ, SA (Barberá del Vallés, Barcelona, Spain). 1-Octadecene was commercially available from Sigma-Aldrich Química (Madrid, Spain). (*Z*)-1-Bromo-11-hexadecene (**7**) was synthesised from (*Z*)-11-hexadecen-1-ol following a procedure previously described by the present authors.[21] Trifluoromethyl ketones **4** and **6** were prepared from the corresponding alcohol precursors by conversion into the corresponding iododerivatives and subsequent *trans*-metallation reactions followed by treatment with ethyl trifluoroacetate.[19] All reactions involving air- or moisture-sensitive materials were carried out under Ar. All solvents were dried and distilled according to standard procedures. IR spectra were recorded on a Bomem MB-120 (Bomem Inc., Quebec, Canada) or Nicolet Avatar 360 FT-IR spectrometer (Thermo Electron Inc., Madison, WI). NMR spectra were recorded at 300 MHz for ^{1}H, at 75 MHz for ^{13}C and at 282 MHz for ^{19}F on a Varian Unity 300 MHz spectrometer (Varian Inc., Palo Alto, CA). Mass spectra (MS) were obtained on a Fisons MD 800 instrument (Thermo Fisher Scientific, Waltham, MA). Elemental analyses (C, H, N, F) were determined on a Carlo Erba-1108 (Carlo Erba Instruments, Milan, Italy) and on a Metrohm Titrando 808 analyser (Metrohm, Herisau, Switzerland). The electrophysiology tests were carried out on a commercial electroantennogram (EAG) apparatus (Syntech, Hilversum, The Netherlands).

2.2 Synthesis of allylic TFMKs 3 and 5

2.2.1 (*Z*)-12-Heptadecene-1-nitrile (**8**)

A mixture of (*Z*)-1-bromo-11-hexadecene (**7**) (5.50 g, 18.13 mmol), NaCN (1.07 g, 21.76 mmol), *n*-tributylamine (0.25 mL) and H_2O (3.2 mL) was heated at reflux for 4 h, according to the procedure described by Apparu *et al.*[22] The crude product was diluted with brine and extracted with hexane. The combined organic layers were dried with $MgSO_4$ and the solvent was removed under reduced pressure. After purification by column chromatography (silica gel, hexane : ether 95 : 5), the corresponding nitrile **8** (4.25 g, 94%) was obtained as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 5.34 (m, 2H); 2.32 (t, $J = 6.9$ Hz, 2H); 2.01 (m, 4H); 1.65 (m, 2H); 1.35 (bs, 18H); 0.89 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 129.8; 129.7; 119.8; 31.9; 29.7; 29.4; 29.2; 29.2; 28.7; 28.6; 27.1; 26.8; 25.3; 22.3; 17.0; 13.9. IR (film, NaCl) ν: 3005, 2926, 2855, 1465 cm^{-1}. MS (EI) *m/z* (%): 249 (M^+, 5); 206 (11); 136 (40); 122 (54); 97 (24); 83 (32); 69 (52); 55 (100); 41 (40). Elemental analysis: Calcd for $C_{17}H_{31}N$: C, 81.86; H, 12.53; N, 5.62. Found: C, 82.04; H, 12.46; N, 5.45.

2.2.2 (*Z*)-12-Heptadecenal (**9**)

A similar procedure to that described by Villuendas *et al.*[19] was used. Thus, a mixture of (*Z*)-12-heptadecene-1-nitrile (4.25 g, 17.04 mmol), 1 M DIBAH solution in hexane (20.45 mL, 20.45 mmol) and anhydrous hexane (100 mL) was stirred at −78 °C under Ar. After stirring for 5 h at −78 °C, the reaction mixture was allowed to warm to 0 °C and NH_4Cl saturated solution (200 mL) was added. The organic phase was decanted and the aqueous layer was extracted with hexane. The aqueous phase was acidified with 1 M HCl and extracted with diethyl ether. The combined organic phases were dried with $MgSO_4$ and the solvent was removed under vacuum. The residue was purified by column chromatography on silica gel, eluting with hexane : ether (95 : 5), to obtain aldehyde **9** (3.48 g, 81%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 9.75 (t, $J = 1.8$ Hz, 1H); 5.34 (m, 2H); 2.41 (td, $J_1 = 7.2$ Hz, $J_2 = 1.8$ Hz, 2H); 2.01 (m, 4H); 1.62 (m, 2H); 1.30 (bs, 18H); 0.89 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 202.9; 129.8; 43.9; 31.9; 29.7; 29.5; 29.5; 29.4; 29.3; 29.2; 29.1; 27.1; 26.9; 22.3; 22.0; 14.0. IR (film, NaCl) ν: 3005, 2926, 2854, 1728, 1465 cm^{-1}. MS (EI) *m/z* (%): 234 (3); 98 (26); 95 (28); 83 (31); 81 (35); 69 (43); 67 (33); 55 (100); 43 (24); 41 (42). Elemental analysis: Calcd for $C_{17}H_{32}O$: C, 80.88; H, 12.78. Found: C, 80.97; H, 12.58.

2.2.3 (*Z*)-1,13-Octadecadiene (**10**)

To a suspension of methyltriphenylphosphonium bromide (1.25 g, 3.51 mmol) in anhydrous THF (10 mL) was added 1.4 M of *n*-BuLi solution in hexane (2.2 mL, 3.07 mmol). The resulting orange solution was stirred for 1 h and cooled to −78 °C, and a solution of (*Z*)-12-heptadecenal (0.50 g, 1.98 mmol) in THF (5 mL) was then slowly added. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. The solvent was evaporated, and the crude product was treated with NH_4Cl saturated solution (50 mL) and extracted with hexane. The combined organic layers were washed with brine and dried ($MgSO_4$). After removal of the solvent, purification by column chromatography on silica gel, eluting with hexane, afforded diene **10** (0.39 g, 80%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 5.82 (ddt, $J_1 = 17.1$ Hz, $J_2 = 10.2$ Hz, $J_3 = 6.6$ Hz, 1H); 5.35 (m, 2H); 4.99 (ddt, $J_1 = 17.1$ Hz, $J_2 = 2.4$ Hz, $J_3 = 1.5$ Hz, 1H); 4.93 (ddt, $J_1 = 10.2$ Hz, $J_2 = 2.4$ Hz, $J_3 = 1.2$ Hz, 1H); 2.02 (m, 6H); 1.33 (bs, 20H); 0.90 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 139.3; 129.9; 129.8; 114.1; 33.8;

32.0; 29.8; 29.6; 29.5; 29.5; 29.3; 29.1; 28.9; 27.2; 26.9; 22.3; 14.0. IR (film, NaCl) ν: 3005, 2924, 2854, 1465, 909 cm^{-1}. MS (EI) *m/z* (%): 250 (M^+, 4); 96 (42); 82 (54); 69 (40); 55 (100). Elemental analysis: Calcd for $C_{18}H_{34}$: C, 86.32, H, 13.68. Found: C, 86.30; H, 13.66.

2.2.4 (E,Z)-1,1,1-Trifluoro-4,15-eicosadien-2-ol (**12**)

To a solution of diene **10** (2.50 g, 9.98 mmol) in anhydrous CH_2Cl_2 (10 mL) was added trifluoroacetaldehyde ethyl hemiacetal (1.2 mL, 9.98 mmol), followed by slow addition of $BF_3{\cdot}OEt_2$ (3.8 mL, 29.94 mmol).[23] The mixture was stirred at room temperature for 4 h, treated with 10% HCl and ice and extracted with CH_2Cl_2. The combined organic layers were washed with brine and dried ($MgSO_4$), and the solvent was removed. The crude product was purified by column chromatography on silica gel, eluting with hexane : ether 98 : 2, to afford the corresponding diunsaturated trifluoromethylcarbinol **12** (2.61 g, 75%) with an 86:14 *E* : *Z* ratio. Stereochemically pure *E* isomer was obtained through a second column chromatography on 10% $AgNO_3$-impregnated silica gel,[24] eluting with hexane : ether (98 : 2), to obtain stereomerically pure (*E,Z*)-1,1,1-trifluoro-4,15-eicosadien-2-ol (**12**) (2.20 g, 63%). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 5.65 (dtt, J_1 = 15.3 Hz, J_2 = 6.9 Hz, J_3 = 1.2 Hz, 1H); 5.37 (m, 3H); 3.93 (m, 1H); 2.38 (m, 2H); 2.17 (d, J = 6.0 Hz, 1H); 2.03 (m, 6H); 1.31 (bs, 18H); 0.90 (t, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 136.8; 129.9; 124.9 (q, J = 281 Hz, –CH(OH)CF_3); 122.4; 69.7 (q, J = 31 Hz, –*C*H(OH)CF_3); 33.3; 32.5; 31.9; 29.7; 29.5; 29.5; 29.4; 29.3; 29.2; 29.1; 27.2; 26.9; 22.3; 14.0. ^{19}F NMR (282 MHz, $CDCl_3$), δ (ppm): −79.8 (d, J = 6.5 Hz, CH(OH)CF_3). IR (film, NaCl) ν: 3404, 3006, 2930, 2853, 1466, 1278, 1173, 969 cm^{-1}. MS (EI) *m/z* (%): 348 (M^+, 19); 208 (29); 166 (79); 152 (20); 139 (41); 123 (39); 109 (79); 97 (77); 95 (90); 81 (92); 69 (87); 67 (94); 55 (100); 43 (65); 41 (96). Elemental analysis: Calcd for $C_{20}H_{35}F_3O$: C, 68.93; H, 10.12; F, 16.36. Found: C, 68.90; H, 10.17; F, 16.49.

2.2.5 (E,Z)-1,1,1-Trifluoro-4,15-eicosadien-2-one (**3**)

To a 15% solution of Dess–Martin periodinane[25] in CH_2Cl_2 (16.82 g, 5.95 mmol) was added dienol **12** (0.83 g, 2.38 mmol) in CH_2Cl_2 (10 mL) under nitrogen. After stirring for 2 h, the reaction mixture was diluted with diethyl ether, and the resulting suspension was treated with 1.5 M NaOH solution and stirred for 10 min. Removal of the solvent and Kugelrohr distillation of the remaining semi-solid gave TFMK **3** (0.70 g, 85%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 5.65 (dtt, J_1 = 15.3 Hz, J_2 = 6.6 Hz, J_3 = 1.2 Hz, 1H); 5.47 (dtt, J_1 = 15.3 Hz, J_2 = 6.6 Hz, J_3 = 1.2 Hz, 1H); 5.35 (m, 2H); 3.42 (d, J = 6.6 Hz, 2H); 2.02 (m, 6H); 1.33 (bs, 18H); 0.89 (t, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 189.8 (q, J = 35 Hz, –*C*OCF_3); 137.8; 129.9 (2×); 117.6; 115.6 (q, J = 291 Hz, –COCF_3); 40.0; 32.5; 32.0; 29.7; 29.5; 29.5; 29.4; 29.3; 29.1; 28.9; 27.2; 26.9; 22.3; 14.0. ^{19}F NMR (282 MHz, $CDCl_3$), δ (ppm): −79.2 (s, COCF_3). IR (film, NaCl) ν: 3006, 2926, 2854, 1766, 1466, 1209, 1148, 1005, 967, 708 cm^{-1}. MS (EI) *m/z* (%): 346 (M^+, 4); 277 (4); 138 (16); 97 (17); 96 (22); 95 (28); 83 (31); 82 (31); 81 (36); 69 (43); 55 (100); 43 (24); 41 (39). Elemental analysis: Calcd for $C_{20}H_{33}F_3O$: C, 69.33, H, 9.60; F, 16.45. Found: C, 69.19; H, 9.69; F, 16.53.

2.2.6 (E)-1,1,1-Trifluoro-4-eicosen-2-ol (**13**)

This compound was obtained as previously described for dienol **12**. Starting from 1-octadecene (**11**) (0.50 g, 1.98 mmol), trifluoroacetaldehyde ethyl hemiacetal (0.23 mL, 1.98 mmol) and $BF_3{\cdot}OEt_2$ (0.75 mL, 5.94 mmol) in anhydrous CH_2Cl_2 (3 mL), trifluoromethyl carbinol **13** (0.52 g, 75%) was obtained in an 88:12 *E* : *Z* ratio after purification by column chromatography (silica gel, hexane : ether 98 : 2). Stereochemically pure *E,Z* isomer (0.42 g, 61%) was obtained after column chromatography on silica gel impregnated with 10% $AgNO_3$,[24] eluting with hexane : ether (98 : 2). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 5.65 (dtt, J_1 = 15.3 Hz, J_2 = 6.6 Hz, J_3 = 1.2 Hz, 1H); 5.40 (dt, J_1 = 15.3 Hz, J_2 = 6.9 Hz, 1H); 3.92 (m, 1H); 2.38 (m, 3H); 2.04 (m, 2H); 1.32 (bs, 26H); 0.88 (t, J = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 136.8; 124.9 (q, J = 280 Hz, CH(OH)CF_3); 122.5; 69.7 (q, J = 31 Hz, *C*H(OH)CF_3); 33.3; 32.6; 31.9; 29.7; 29.7; 29.6; 29.5; 29.4; 29.2; 29.2; 22.7; 14.1. ^{19}F NMR (282 MHz, $CDCl_3$), δ (ppm): −79.8 (d, J = 6.8 Hz, CH(OH)CF_3). IR (film, NaCl) ν: 3406, 3004, 2922, 2853, 1463, 1277, 1170, 1134, 970, 909, 733, 698 cm^{-1}. MS (EI) *m/z* (%): 350 (M^+, 2); 139 (19); 125 (19); 111 (39); 97 (69); 83 (83); 69 (51); 57 (100); 55 (79); 43 (74); 41 (51).

2.2.7 (E)-1,1,1-Trifluoro-4-eicosen-2-one (**5**)

This compound was obtained as previously described for TFMK **3.** Starting from dienol **13** (2.52 g, 7.19 mmol) and a 15% solution of Dess–Martin reagent[25] in CH_2Cl_2 (51.00 g, 17.97 mmol), TFMK **5** (2.16 g, 86%) was obtained after Kugelrohr distillation as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 5.65 (dtt, J_1 = 15.3 Hz, J_2 = 6.6 Hz, J_3 = 1.2 Hz, 1H); 5.47 (dt, J_1 = 15.3 Hz, J_2 = 6.6 Hz, 1H); 3.42 (d, J = 6.6 Hz, 2H); 2.06 (m, 2H); 1.32 (bs, 26H); 0.88 (t, J = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 189.7 (q, J = 34 Hz, *C*OCF_3); 137.8; 117.6; 115.6 (q, J = 291 Hz, COCF_3); 40.0; 32.6; 32.0; 29.7; 29.6; 29.5; 29.4; 29.1; 29.0; 22.7; 14.1. ^{19}F NMR (282 MHz, $CDCl_3$), δ (ppm): −79.2 (s, COCF_3). IR (film, NaCl) ν: 3004, 2926, 1765, 1463, 1208, 1151, 1006, 968 cm^{-1}. MS (EI) *m/z* (%): 348 (M^+, 1); 279 (11); 138 (100); 97 (45); 83 (59); 69 (64); 57 (95); 55 (24); 43 (72); 41 (40). Elemental analysis: Calcd for $C_{20}H_{35}F_3O$: C, 68.93; H, 10.12; F, 16.36. Found: C, 69.04; H, 10.31; F, 16.23.

2.3 Adults of *Zeuzera pyrina*

Adults of *Z. pyrina* were obtained from a population originated from larvae collected in the fruit-growing area of Lleida (north-eastern Spain). The larvae were reared on a semi-synthetic diet[26] at 25 ± 5 °C under a 16 : 8 h light : dark photoperiod at the Centre UdL-IRTA for R&D (Lleida, Spain). The larvae were kept in cylindrical plastic boxes in groups of five during the first-development instars, and they were isolated during their last two instars. Pupae were separated by sex, and males and females were kept under the above-mentioned conditions in different environmental chambers until adult emergence. Males and females less than 4 days old were used in the experiments, as in previous unpublished studies the authors had found that *Z. pyrina* females only called and males were only active during the first 3 days after emergence.

2.4 TFMK activity assays

2.4.1 Electrophysiology

To test the inhibitory effect of the TFMKs, the response to pheromone stimuli of antennae previously treated with TFMKs was compared with the response of untreated antennae of the same individual. Twelve males were slightly anaesthetised with ice for 5–10 min, and a hexane solution (0.5 µL, 0.02–2 µg µL^{-1}) of TFMK **3** or **4** or hexane alone was topically applied to one of their antennae. After 5 min at room temperature to allow males to recover, the antennae were excised and their last segments were cut. The antennae were then fixed to the electrodes with conducting gel Spectra 360 (Parker Lab. Inc., Hellendoorn, The

Netherlands). A flow of humidified pure air (1000 mL min^{-1}) was continuously directed over the male antenna through the main branch of a glass tube (7 cm long × 5 mm diameter). Test stimuli were carried out by giving 8–10 puffs of air (300 mL min^{-1}) for 100 ms at 40 s intervals through a Pasteur pipette with a CS-01 stimulus controller (Syntech). The pipette contained a small piece of filter paper (2 × 1 cm) on which 10 µg of the pheromone blend had been deposited. This amount was chosen after previous experiments had shown that it provoked, in the present conditions, a response of 1.57 ± 0.33 mV ($n = 22$, unpublished data), the highest mean response observed in the range tested (0.3–30 µg of pheromone). Control puffs with a piece of paper containing only solvent (hexane) were interposed between two consecutive puffs to determine the baseline depolarisation of the antennae. The signals were amplified (100×) and filtered (DC to 1 kHz) with an IDAC-2 interface (Syntech), digitised on a PC and analysed with the EAG Pro program. The response inhibition of each TFMK and dose tested was calculated as the percentage of the response of the treated antenna relative to that of the untreated one. The inhibition values were analysed by one-way ANOVA followed by a Duncan's multiple range test ($P < 0.05$), using the SAS system (v.8).

2.4.2 *Wind tunnel*

The experiments were carried out in glass wind tunnels previously described,[27,28] located at the Spanish National Research Council in Barcelona and at the University of Lleida (Spain).

2.4.2.1 Bioassay procedure

The general conditions of the experiments were: average temperature inside the tunnel, 23 ± 2 °C; relative humidity, 60%; illumination with red light bulbs, 1–3 lux; wind speed, 0.2 m s^{-1}; time of day, from the beginning of the scotophase to 2 h later. The males were maintained in the wind tunnel room for 30 min prior to the experiments for acclimatisation. The males were individually placed on a 25 cm high platform that was placed 130 cm apart from the emission source. They were allowed 10 min to respond to the stimulus, and four behavioural responses were recorded: take-off (TO: the male left the platform and started to fly); half-way (HW: upwind flight until arrival to the middle of the wind tunnel); complete flight (CF: upwind flight to the proximity of the source); source contact (SC: touchdown with the emission source). Twenty-two males were used for the experiments. Each male was used several times owing to the difficulties of obtaining adults in the laboratory. No statistical analysis was carried out, therefore, as the samples were not independent.

2.4.2.2 Pheromone assays

To determine the male response to the sex pheromone, males were flown to a virgin calling female that had been placed inside a metal cage at the same height as the insect release platform.

2.4.2.3 Inhibition assays

Only the effect of TFMK **4** was investigated. For the tests, different amounts (0.1, 1, 5 and 10 µg) of TFMK **4** were deposited on a filter paper (2 × 2 cm) that was placed 1 cm apart from the calling female. Each male was flown first only to the calling female and to both the female and the inhibitory component simultaneously afterwards.

2.4.2.4 Flight tracks

Tracks of flying insects were recorded with a CCD 2400JB Presentco video camera (Rister, Barcelona, Spain) equipped with a 12 mm wide-angle lens. The camera was mounted at a height of 140 cm above the tunnel in a perpendicular position to record the insect flight with a minimal optical distortion. The camera covered a 130 × 45 cm section of the tunnel, and the recorded tracks were sent to a monitor for visualisation. The tracks were converted into computer files at a rate of 25 frame s^{-1} with the aid of digital video software (Pinnacle Systems 5.1, Mountain View, CA). The successive insect positions were converted into *XY* coordinates using in-house software, and they were plotted in graphs at 0.4 s intervals. Only uninterrupted flights were considered.

2.4.3 *Field tests*

Field tests were carried out in 2003 and 2004 in infested apple and olive orchards in the fruit-growing areas of Lleida and Girona (Catalonia, north-eastern Spain) during the flight period of *Z. pyrina* males (from mid-May to early September). Mixtures of the pheromone and compounds **3, 4, 5** and **6** at 1 : 1 and 1 : 5 ratios were obtained by adding the required amount of the inhibitor to 5 mg of the pheromone. Dispensers containing only 5 mg of the pheromone were used as controls. Polyethylene vials and 36 × 20 cm delta traps were used. Traps were hung over the canopy of the crop and spaced 20 m from each other; the dispensers and the traps were changed once during the tests. The position of the traps was rotated once per week, and the trials finished after two complete rotations of trap position had been carried out. Three replicates per formulation were carried out. A fully randomised block design was used. The total number of catches was treated with a one-way ANOVA, and the mean number of catches was compared with a Duncan's multiple range test ($P < 0.05$) using the SAS system (v.8).

3 RESULTS AND DISCUSSION

3.1 Synthesis

The main features of the major pheromone compound of the leopard moth are the presence of two unsaturations at positions 2 and 13 in the aliphatic chain. Therefore, it was essential for the TMKs considered as possible inhibitors to contain both double bonds at the same position and with the same stereochemistry.

Although several methods have been developed for the synthesis of TFMKs,[19,20,29–34] in the case of allylic TFMKs **3** and **5** new problems arose owing to the enhanced reactivity of the allylic methylene group in the α-position to the carbonyl. For preparation of these compounds, the authors initially tried to introduce the double bond at position 3 by desulfonation of the α-phenylsulfonyl derivative, but preparation of this compound failed.[18] Also, reaction of the carboxylic acid precursor with an electrophile[31] or *trans*-metallation of the corresponding 1-iodo-2-alkadiene with *tert*-butyllithium[19] were unsuccessful. The only precedent found in the literature for the synthesis of these TFMK precursors was reported by Sakumo *et al.*,[23] who prepared fluorinated homoallyl alcohols by adding olefins to trifluoroacetaldehyde hemiacetal through an ene-type reaction. These authors used this approach to obtain trifluoromethylated dienes through dehydration of trifluoromethyl homoallyl alcohols,[35,36] and only in one case did they apply this methodology to prepare an allylic TFMK (1,1,1-trifluoro-4-dodecen-2-one).[37]

The strategy followed for the preparation of TFMKs **3** and **5** is depicted in Fig. 2. Transformation of (*Z*)-1-bromo-11-hexadecene

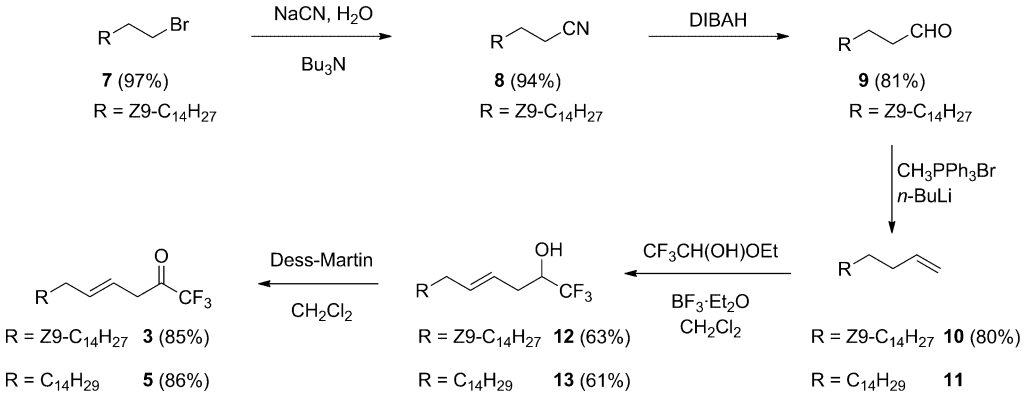

Figure 2. Synthesis of allylic trifluoromethyl ketones **3** and **5**.

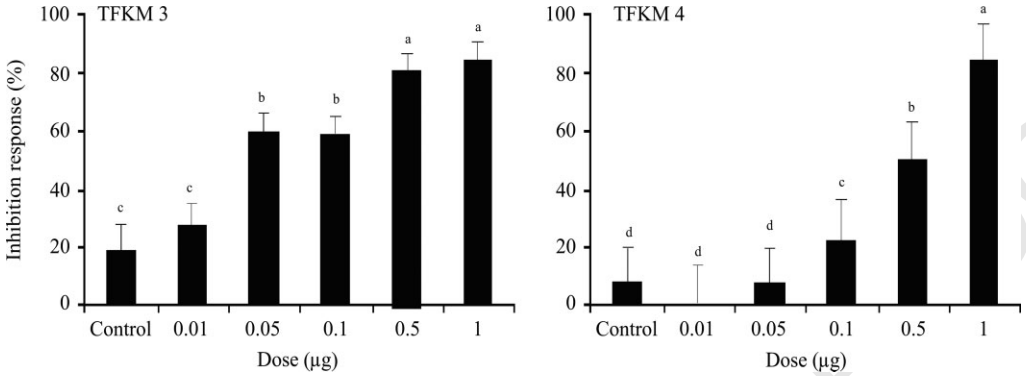

Figure 3. Inhibition of the EAG response of *Zeuzera pyrina* males to the sex pheromone after topical application of different doses of TFMK **3** (left) or TFMK **4** (right). Bars with the same letter are not significantly different (Duncan's multiple range test, $P < 0.05$).

(**7**) into nitrile **8** was accomplished by reaction with aqueous sodium cyanide in the presence of tributylamine as phase transfer catalytic agent.[22] Compound **8** was reduced to aldehyde **9** under standard conditions (DIBAH/hexane), and Wittig reaction of **9** with methyltriphenylphosphonium ylide afforded diene **10** in good yield. At this point, diene **10** and commercial 1-octadecene (**11**) were used as starting materials for the ene reaction with trifluoroacetaldehyde ethyl hemiacetal and boron trifluoride etherate as Lewis acid. After 4 h at room temperature, the reaction was completed, affording the desired homoallyl alcohols **12** and **13** in 63% and 61% yields respectively. Under these conditions, the disubstituted double bond of **12** at position 13 remained untouched, pointing out the regioselective character of the reaction. Another important point is the diastereoselectivity of this reaction. The process occurs through an anti-elimination mechanism to form the *E* isomer predominantly. The *E* : *Z* isomeric ratio of the new double bond formed was 86 : 14 for alcohol **12** and 88 : 12 for alcohol **13**. This remarkable diastereoselectivity made it possible to obtain the stereochemically pure (≥98%) *E* isomers by column chromatography on $AgNO_3$-impregnated silica gel. In addition, an excellent 98% of the *E* isomer was recovered. Finally, mild oxidation of alcohols **12** and **13** with the Dess–Martin reagent afforded allylic TFMKs **3** and **5** in 85–86% yields.

Homoallylic TFMK **4** was also prepared because it was closely structurally related to the minor pheromone component **2.** Monoene TFMK **6**, in turn, was considered to evaluate the importance of the unsaturation at position 2 or 3 on the inhibition activity. Both compounds **4** and **6** were obtained following a procedure previously described by the present authors.[19] The retention times of the pheromone components **1** and **2** and the TFMKs **3** to **6** on an HP5 column (30 m × 0.25 mm × 0.25 µm) (GC conditions: injection at 120 °C for 1 min and programmed rise in temperature at 3 °C min^{-1} until 280 °C for 10 min) were: (*E*,*Z*)-**1**, 32.33 min; (*E*,*Z*)-**2**, 31.86 min; (*E*,*Z*)-**3**, 26.18 min; (*E*,*Z*)-**4**, 24.55 min; (*E*)-**5**, 26.58 min; (*Z*)-**6**, 24.87 min.

3.2 TFMK activity

3.2.1 Electrophysiology

When TFMKs **3** and **4** were electrophysiologically tested, both compounds showed a remarkable inhibition of the electroantennogram (EAG) responses to the pheromone (Fig. 3). In both cases the inhibition was dose dependent, and it reached values greater than 80% at the highest dose tested (1.0 µg $antenna^{-1}$). The effect of **3**, the analogue of the major pheromone compound, was stronger than the effect of **4**, the analogue of the minor pheromone compound. The inhibition caused by **3** was significantly different from the control (hexane) for doses of ≥0.05 µg $antenna^{-1}$, and the maximum inhibition was obtained at 0.5 and 1.0 µg $antenna^{-1}$, whereas for **4** the inhibition was significantly different from the control (hexane) for doses 20 times greater than for **3** (0.1 µg $antenna^{-1}$), and the maximum inhibition was obtained at a dose of 1 µg $antenna^{-1}$. Similar results were obtained on *Spodoptera littoralis* Boisduval, where 3-octylthio-1,1,1-trifluoropropan-2-one (OTFP) decreased the EAG amplitude and increased the repolarisation time of the pheromone response, the EAG kinetics being increased with the TFMK dose.[38] Also, exposure of antennal receptors of *Spodoptera frugiperda* (JE Smith) to vapours of OTFP resulted in a decreased amplitude and 2/3

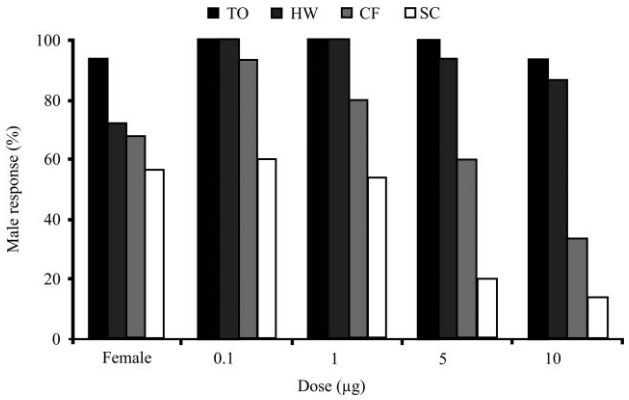

Figure 4. Response of *Zeuzera pyrina* males to the calling female ($n = 22$) and to the calling female in the presence of different amounts of TFMK **4** ($n = 15$). TO: take-off; HW: half-way; CF: complete flight; SC: source contact.

repolarisation time of the EAG response to the pheromone, the effect being reversible.[39] The non-fluorinated analogue (OTP) did not affect any of the EAG parameters, confirming the key role played by fluorine atoms in this type of chemical.[39,40] In the European corn borer *Ostrinia nubilalis* (Hübner), the TFMK analogue of the major component of the pheromone inhibited the EAG response to the natural attractant in a dose–response manner, and 10 pg of the ketone was sufficient to promote a 77% inhibition response.[40]

3.2.2 *Wind tunnel*

In 95% of the cases, males took off in response to the calling female, but only in 62% of cases did they show an oriented flight towards the source, and in only 55% did they contact the female (Fig. 4). Unpublished results from the authors' group have shown that *Z. pyrina* males do not fly easily in the wind tunnel, their behaviour being highly variable (a variable number of contacts have been recorded of males tested under almost identical conditions). Nevertheless, the percentage of complete upwind flights obtained in the experiments reported here (55%) is high enough to detect a possible disruptant effect of the TFMKs. Although no statistical analysis could be performed, as the same males were used several times in the experiments, a change in their behaviour towards the calling females in the presence of amounts of TFMK **4**, the analogue of the minor component of the pheromone, equal to or higher than 5 µg was evident (Fig. 4). TFMK **4** decreased the percentage of males that showed a complete flight and contacted the female. The inhibition took place after the male had passed the middle of the tunnel, i.e. in the CF and SC behaviour (Fig. 4). The disrupting effect exerted by TFMK **4** was also evident when the flight tracks were video-recorded. In the presence of the inhibitor, males displayed an erratic flight (or less oriented in the presence of the lower dose of the TFMK, flight A versus flight B in Fig. 5) to the source, in contrast to the much more oriented flight onto the plume that was shown by control insects (flight C versus flight D in Fig. 5). Consequently, flight tracks were longer when the TFMK **4** was present in the lure than when the female was alone. These results agree with those previously obtained on *Sesamia nonagrioides* Lefevre[16] and *O. nubilalis*,[41] when males were attracted to mixtures of the TFMK analogues of the pheromone and the natural attractant in 5 : 1 and 10 : 1 ratios, confirming the disruptant effect of these chemicals in different moth species.

3.2.3 *Field tests*

The activity of the synthetic compounds in the field was determined by evaluating the number of insects caught with blends of pheromone : TFMK. In 2003, the dienic TFMK **4** displayed the highest inhibition activity among the three compounds tested (**4** to **6**) (Fig. 6). Compound **6**, which lacks the unsaturation at position 2, was a stronger inhibitor than **5**, which lacks the unsaturation at position 13, and the difference was significant at the two ratios tested. These results were confirmed at a pheromone : inhibitor ratio of 1 : 1 in the 2004 trial at Lleida Fuliola (Fig. 7). From these results, the conclusions drawn are twofold: firstly, the most closely related analogue of one of the pheromone components is the best inhibitor; secondly, the presence of the double bond at position 13 plays a crucial role in the activity of

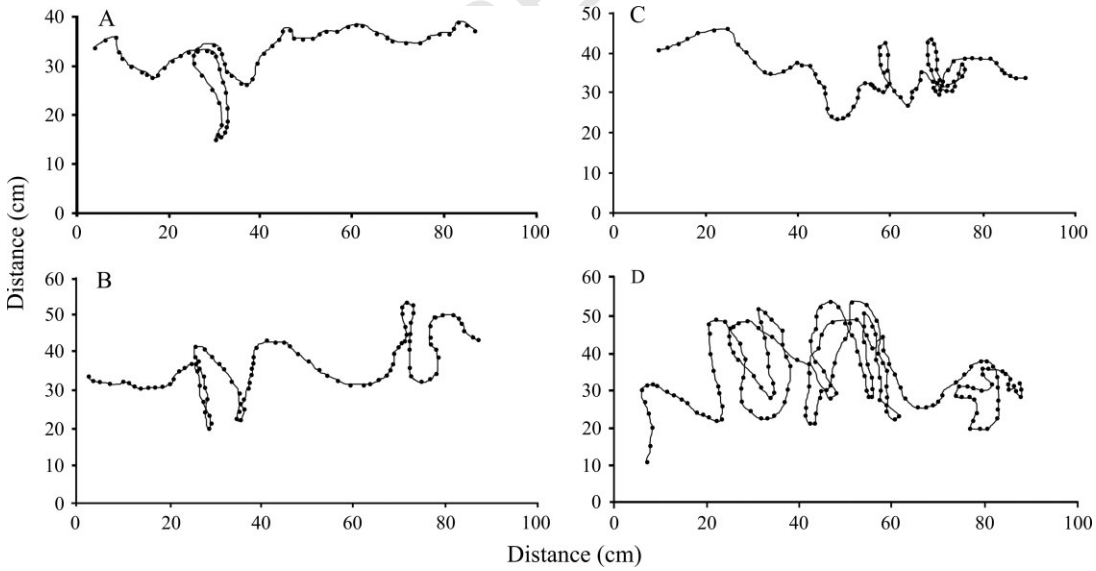

Figure 5. Track flights of *Zeuzera pyrina* males towards a calling female (A and C) and towards a calling female in the presence of TFMK **4** at 0.1 µg (B) and at 1 µg (D). The insect moved upwind from left to right. The insect position at 0.4 s intervals is shown by the dark points.

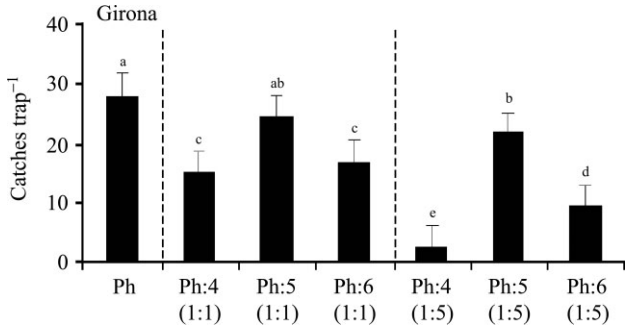

Figure 6. Field catches of *Zeuzera pyrina* males in traps placed on infested olive trees in Girona in 2003. Traps were baited with mixtures of pheromone and TFMKs **4** to **6** in 1 : 1 and 1 : 5 ratios. The amount of pheromone in each trap was 5 mg. Bars with the same letter are not significantly different (Duncan's multiple range test, $P < 0.05$).

these chemicals. In 2004, the authors compared the activity of the dienic compounds **3** and **4** in three field trials. Except in one case, the addition of TFMKs **3** and **4** reduced significantly the total number of catches, and reductions higher than 90% were achieved (Fig. 7). However, it was not clear whether compound **3**, the analogue of the major component of the pheromone, was a stronger inhibitor than **4**, the analogue of the minor component. The high variability of the number of catches probably prevented the detection of significant differences. The observed trend was different in Girona, where **3** seemed to be more effective than in Lleida, where **4** appeared to be more effective. A mixture of TFMKs **3** and **4** in the same ratio (96 : 4) as the corresponding components in the pheromone blend did not result in a significant difference in activity in comparison with the major analogue **3** alone. The present field results confirm the antagonist activity of other TFMK analogues of the pheromone that was previously recorded on *S. nonagrioides*,[16] *O. nubilalis*[40,41] and *Cydia pomonella* (L.).[17] Moreover, in large-scale field experiments, application of (*Z*)-11-hexadecenyl trifluoromethyl ketone, an analogue of the *S. nonagrioides* pheromone, yielded a noticeable reduction in damage caused by this pest and by the sympatric species *O. nubilalis*, both in the number of plants attacked and in the number of larvae found per plant.[42]

Pheromone inhibitors, as analogues of the natural pheromones, have been used in mating disruption experiments for pest control.[43] The present authors have demonstrated in recent years that TFMKs are potent antagonists of the pheromone in the field and good inhibitors in electrophysiology and behaviour in the laboratory.[16,17,38–42] In addition, although it has not been determined in antennal extracts of *Z. pyrina*, these chemicals displayed a remarkable antiesterase activity in *S. littoralis*,[11,14] *S. nonagrioides*[44] and *O. nubilalis*.[38] Antennal esterases are key enzymes for the rapid degradation of pheromone esters, and inhibition of these enzymes would induce saturation or blocking of pheromone receptors, leading to a decreased capability of the insect to detect new incoming pheromone molecules. TFMKs can also bind to pheromone-binding proteins (PBPs) in competition with pheromone molecules,[45,46] as shown in binding experiments by displacement of the major component of the pheromone of *Mamestra brassicae* Linné by (*Z*)-11-16:trifluoromethyl ketone.[47] TFMKs have shown low toxicity in Swiss mice, e.g. (*Z*)-11-hexadecenyl trifluoromethyl ketone and OTFP displayed an LD_{50} of 1 g kg^{-1} body weight after 6 days of treatment, whereas the pheromone showed an LD_{50} of 5 g kg^{-1} body weight.[16] These data agree with a previous report in which doses of up to 250 mg kg^{-1} body weight of some fluorinated ketones for over 3 months caused no mortality on Swiss Webster mice.[48] Also, it has been found in aquatic ecotoxicity studies that (*Z*)-11-hexadecenyl trifluoromethyl ketone was only moderately toxic, with EC_{50} values ranging from 3.11 to 103.7 mg L^{-1} in algae growth, and from 0.07 to 1.2 mg L^{-1} in *Daphnia* survival.[49] The low toxicity elicited by these compounds is possibly due to their reversible inhibition mechanism, in contrast to the much higher toxicity displayed by other irreversible inhibitors of carboxylesterases and proteases. The results accumulated so far suggest that new studies on the threshold activity of TFMKs are worthwhile in order to evaluate the real prospects of their use as new possible pest control agents.

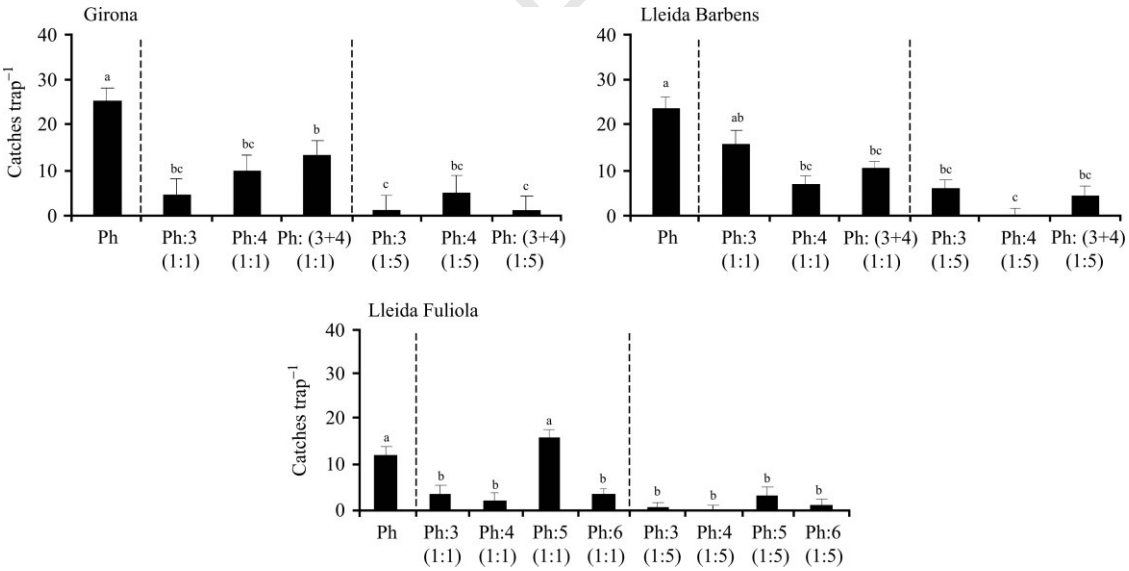

Figure 7. Field catches of *Zeuzera pyrina* males in traps placed on infested apple and olive trees in Lleida and Girona, respectively, in 2004. Traps were baited with mixtures of pheromone and TFMKs **3** to **6** in 1 : 1 and 1 : 5 ratios. The amount of pheromone in each trap was 5 mg. Bars with the same letter are not significantly different (Duncan's multiple range test, $P < 0.05$).

4 CONCLUSION

Efficient syntheses of allylic TFMKs, analogues of the two components of the pheromone of the leopard moth, have been accomplished in good yields, regiospecificity and diastereoselectivity. The most closely related analogues of both pheromone components are good inhibitors of the natural attractant both in the laboratory and in the field. The presence of an unsaturation at positions 2 and 13 in the structure of these analogues is crucial for optimum activity.

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